ENVIRONMENT, WELL-BEING, AND BEHAVIOR

Stocking Density and Physiological Adaptive Responses of Broilers[1]

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ABSTRACT Three trials were conducted to assess the effects of stocking density on physiological adaptive responses of broilers. Male broilers were reared in floor pens under conditions similar to those used commercially in the United States. Accepted indicators of adaptation to a stressor were measured on d 49 including plasma concentrations of corticosterone, glucose, cholesterol, and total nitrites as an indicator of nitric oxide, as well as heterophil:lymphocyte ratio. In trial 1, calculated stocking densities were 20, 25, 30, 35, 40, 45, 50, and 55 kg of BW/m^2 and in trials 2 and 3, stocking densities were 30, 35, 40, and 45 kg of BW/m^2. Stocking densities were calculated based on a final BW of 3.3 kg. Linear trend analyses were used to assess the role of stocking density on each of the physiological parameters. Results indicate that stocking density did not cause physiological adaptive changes indicative of stress.

Key words: stocking density, corticosterone, glucose, cholesterol, heterophil:lymphocyte ratio

INTRODUCTION

Today, much concern is expressed about stocking density as it relates to the well-being and welfare of broilers. A large part of this concern centers on the question of whether stocking density causes adaptive responses that are characteristic of physiological stress.

For many years, the term stocking density indicated the numbers of birds being reared in a given housing area (Rice and Botsford, 1925). Today, broilers are reared to target BW that far exceed those of only a decade ago. To account for these large increases in final BW, it has become necessary to develop a new expression of stocking density. Specifically, many in the poultry industry now express stocking density as mass per unit of space. This expression of stocking density is calculated based on body mass (in kg or lb) per unit of housing space (in m^2 or ft^2).

Several reports have been published recently describing the effects of stocking density expressed as mass per unit of space, on production characteristics, including BW gain, yield, feed consumption, feed conversion, and mortality (Puron et al., 1995; Feddes et al., 2002; Dawkins et al., 2004). Additionally, various behavioral aspects of broilers have been related to stocking density (Murphy

and Preston, 1988; Newberry and Hall, 1988; Preston and Murphy, 1989; Lewis and Hurnik, 1990; Bessei, 1992; Bessei and Reiter, 1992; Andrews et al., 1997). Several morphological anomalies have also been presented, indicating that increased stocking density can compromise immunity (Proudfoot et al., 1979; Greene et al., 1985; McIlroy et al., 1987; Heckert et al., 2002).

Little has been published concerning the influence of stocking density on physiological adaptive responses. Dawkins et al. (2004) surveyed 2.7 million broilers maintained under commercial conditions by 10 companies in the United Kingdom. These birds were reared under stocking densities between 30 and 46 kg of BW/m^2. They assayed corticosterone (**CS**) levels in feces and did not find a significant correlation between fecal CS levels and stocking density. Information is lacking concerning blood concentrations of CS, glucose (**GLU**), cholesterol (**CHOL**), and total nitrites, as well as heterophil:lymphocyte ratio (**H:L**), as they relate to stocking density.

Puvadolpirod and Thaxton (2000a,b,c,d) and Post et al. (2003) have proposed models to study physiological adaptive stress responses in broilers. They infused adrenocorticotropin (**ACTH**) via osmotic pumps or fed CS. The ACTH treatment caused the adrenal glands to secrete maximum amounts of CS; feeding CS ensured the same result. Both stress models resulted in 42 different adaptive responses. Thaxton and Puvadolpirod (2000) developed a stress scoring method to compare all of the adaptive responses that occurred in broilers receiving ACTH by infusion. Using this method, they found that the top 10 adaptive changes of broilers are 1) plasma CS, 2) plasma GLU, 3) liver lipid, 4) relative liver weight, 5) total white

blood cell count, 6) protein digestion, 7) total excreta, 8) plasma high-density lipoproteins, 9) water intake, and 10) plasma CHOL. Therefore, the objective of this study was to assess 5 major physiological adaptive responses in broilers reared at varying stocking densities (expressed as final BW/m^2) in housing conditions similar to those presently used to rear broilers commercially in the United States.

MATERIALS AND METHODS

Husbandry

Three trials were conducted using male Ross (Aviagen, Inc., Huntsville, AL) × Cobb (Cobb-Vantress, Inc., Siloam Springs, AK) chicks obtained from a commercial hatchery. Chicks were vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis at the hatchery. The birds were reared in a metal building with solid side walls. A description of the rearing facility, as well as all management procedures, was described in a companion paper (Dozier et al., 2005).

Stocking Densities

In trial 1, broilers were reared at a stocking density of 0.065 kg/m^2 from hatching until 35 d of age. Birds then were allotted to 8 stocking densities: 20, 25, 30, 35, 40, 45, 50, and 55 kg/m^2. The number of 35-d-old birds placed in each of 4 pens with a floor space of 4.18 m^2 was 29, 36, 44, 51, 58, 65, 73, and 80. Birds were maintained from 35 to 49 d at these densities. The expected final BW at 49 d was 3.35 kg.

In trials 2 and 3, 8 pens of broilers were reared at stocking densities of 30, 35, 40, and 45 kg/m^2 from hatching until 49 d of age. The number of birds in each of 4 pens (floor area = 4.18 m^2) was 37, 43, 50, and 56; estimated final BW was 3.29 kg.

Physiological Measurements

In all 3 trials, 8 birds from each pen were selected at random and bled on d 49. Plasma CS, GLU, and CHOL, as well as H:L ratio, were determined on all randomly selected birds from all 3 trials, whereas total nitrites was determined on the selected birds from trials 1 and 2, but not from trial 3.

Care was taken to ensure that the elapsed time between catching a bird and collecting the blood sample did not exceed 45 s. Blood samples were collected using heparinized syringes and the syringe was plunged into an ice bath immediately after each collection. After collection, samples were transported to the laboratory. One drop of whole blood from each sample was expelled to make 2 thin smears on clean microscope slides. The syringes were then centrifuged (5,000 × g for 10 min at 4°C) and the packed cells in each sample were expelled from the syringe. The plasma samples, which remained in the syringes, were stored at −20°C until chemical analyses were

performed. This technique ensured that the plasma samples were never exposed to ambient air.

Plasma CS concentrations were determined by an enzyme-linked immunoassay (**EIA**). The EIA kits (Correlate-EIA for CS, Assay Designs, Inc., Ann Arbor, MI) were based on sheep polyclonal antibody against CS. The mean intra- and interassay coefficients of variation were 4.5 and 3.1%, respectively.

This assay method has been compared directly with RIA. Precision and accuracy of the EIA assay exceeded that of RIA. The EIA procedure involved pipetting 10 μL of each plasma sample into an Eppendorf tube, and adding 10 μL of steroid inhibitor buffer. The solution was then vortexed and all samples were brought to a 1:40 dilution using Tris buffer solution with sodium azide. An aliquot of each sample (100 μL) was pipetted into microtiter plate wells (coated with donkey-anti-sheep IgG) in duplicate. In addition, 50 μL of alkaline phosphatase conjugated with CS and sheep polyclonal antibody was added into each well. After 2 h of incubation on a shaker (500 rpm) at room temperature, plates were washed 3 times in Tris buffer solution containing detergents and sodium azide. A solution of o-nitrophenyl phosphate (200 μL) was added into each well and the plate was incubated for a further hour (without shaking) at room temperature. To stop the reaction, 50 μL of trisodium phosphate solution was added and absorbance was read spectrophotometrically (μQuant Microtiterplate spectrophotometer, Bio-Tek Instruments, Inc., Winooski, VT) at 405 nm. Standard curves and sample concentrations were calculated using the KC Junior Software (Bio-Tek Instruments, Inc.).

Plasma GLU and CHOL were determined using an autoanalyzer that employed colorimetric detection procedures described by Elliot (1984). Total nitrite analysis was performed spectrophotometrically using Griess reagent. A 100-μL aliquot of plasma from each sample was added to each of 2 test tubes containing 500 μL of concentrated sulfosalicylic acid. This mixture was vortexed for 30 s at 5-min intervals for 30 min, and then centrifuged (5,000 × g for 10 min) to remove all denatured proteins. A 100-μL aliquot of supernatant from each of the duplicate extractions of the sample was added to each well of a 96-well, flat-bottomed microtiter plate. Then, 100 μL of Griess reagent was added to each well and the plate was allowed to stand at room temperature for 10 min. Plates were read at 490 nm using a microtiter plate spectrophotometer (Bio-Tek Instruments, Inc.). Concentration of nitrite was determined using a standard curve, as replicated in the original work of Green et al. (1982). All values are expressed as total nitrite in micromoles.

The dried blood smears were stained using Wright's stain and 100 leukocytes were counted microscopically on each smear (Cook, 1959). Heterophil:lymphocyte ratios were calculated and the average for each blood sample was recorded.

Statistical Procedures

All trials were conducted as a randomized complete block design with 4 replicate blocks in trial 1 and 8 repli-

Table 1. Effects of stocking density on plasma corticosterone (CS, pg/mL) concentrations (mean ± SEM) in heavy broilers

Stocking density[1] (kg of BW/m^2)	Trial	
	1	2 and 3
20	493 ± 12	—
25	450 ± 14	—
30	497 ± 15	756 ± 8
35	464 ± 21	784 ± 13
40	466 ± 22	792 ± 14
45	462 ± 14	782 ± 12
50	462 ± 13	—
55	511 ± 14	—
Trend analysis	Probability	
Linear	0.9	0.42
	Estimate	
Slope	−0.4	8.67
Sx of slope[2]	3.17	10.68
LSD (critical values)	61.3	69.09

[1]Stocking density calculated as body mass (kg of BW/m^2) × pen area (4.18 m^2)/estimated final BW (3.35 kg in trial 1, and 3.29 kg in trials 2 and 3).

[2]Sx = standard error of the slope.

Table 2. Effects of stocking density on plasma glucose (GLU, mg/dL) concentration (mean ± SEM) of heavy broilers

Stocking density[1] (kg of BW/m^2)	Trial	
	1	2 and 3
20	244 ± 5	—
25	247 ± 3	—
30	233 ± 8	248 ± 3
35	242 ± 5	254 ± 2
40	244 ± 5	246 ± 2
45	245 ± 7	249 ± 3
50	252 ± 5	—
55	249 ± 7	—
Trend analysis	Probability	
Linear	0.36	0.99
	Estimate	
Slope	1.01	−0.02
Sx of slope[2]	1.07	1.25
LSD (critical values)	21.26	7.82

[1]Stocking density calculated as body mass (kg of BW/m^2) × pen area (4.18 m^2)/estimated final BW (3.35 kg in trial 1, and 3.29 kg in trials 2 and 3).

[2]Sx = standard error of the slope.

cate blocks in trials 2 and 3. Stocking densities were treated as the treatments. Two analyses were conducted: 1) ANOVA followed by LSD comparing stocking density means; and 2) ANOVA using a linear trend to explain stocking density effects. In general, a quadratic trend was not significant ($P \leq 0.05$) for the variables measured in this study. Results of trials 2 and 3 were pooled in both analyses with trial, replication within trial, and trial × replication as random effects. Analyses were performed using PROC MIXED (2004 version, SAS Institute, Inc., Cary, NC). Statements of significance are based on $P \leq 0.05$.

RESULTS

Mean plasma CS concentrations of the stocking density treatments are presented in Table 1. Trend analyses of the 8 densities in trial 1, as well as the 4 densities pooled over trials 2 and 3, showed that stocking density did not result in a recognizable trend in CS concentrations. The CS concentration (mean ± SEM) over all densities in trial 1 was 476 ± 15 pg/mL, and that of trials 2 and 3 was 778 ± 12 pg/mL.

The effects of stocking density on plasma GLU concentrations are presented in Table 2. As with CS concentrations, GLU concentrations did not exhibit a trend in any trial. The mean (± SEM) GLU concentration in birds of trial 1 was 245 ± 6 mg/dL, and that of the combined results of trials 2 and 3 was 249 ± 2 mg/dL.

Table 3 depicts the effects of stocking density on plasma CHOL concentrations in heavy broilers. As with CS and GLU, plasma CHOL concentration was not affected by stocking density. Linear trend analysis showed that a trend relationship between stocking density and CHOL concentration did not occur. Plasma CHOL (mean ± SEM) in birds of trial 1 was 115 ± 5 mg/dL, and that in birds of trials 2 and 3 was 130 ± 3 mg/dL.

Results illustrating the effects of stocking density on total nitrite concentration are presented in Table 4. Linear trend analyses showed that total nitrite concentration over 8 stocking densities in trial 1 and 4 stocking densities in trial 2 did not exhibit a response trend. The total nitrite concentration (mean ± SEM) in birds of trial 1 was 105 ± 6 μM, and in birds of trial 2 it was 73 ± 3 μM.

Results of the effects of stocking density on H:L ratio are presented in Table 5. The birds of trial 1 did not exhibit a linear H:L response trend; however, birds in trials 2 and 3 combined did exhibit a response trend. Specifically, as stocking density increased from 30 to 45 kg of BW/m^2, the H:L ratio increased linearly. The H:L ratio (mean ± SEM) in birds of trial 1 was 1.02 ± 0.16; in birds of trials 2 and 3 it was 1.41 ± 0.11.

Table 3. Effects of stocking density on plasma cholesterol (CHOL, in mg/dL) concentration (mean ± SEM) of heavy broilers

Stocking density[1] (kg of BW/m^2)	Trial	
	1	2 + 3
20	111 ± 3	—
25	108 ± 5	—
30	110 ± 4	124 ± 2
35	116 ± 5	133 ± 2
40	126 ± 7	128 ± 3
45	123 ± 5	135 ± 3
50	116 ± 5	—
55	117 ± 6	—
Trend analysis	Probability	
Linear	0.07	0.07
	Estimate	
Slope	1.39	3.18
Sx of slope[2]	0.75	1.41
LSD (critical values)	61.3	8.08

[1]Stocking density calculated as body mass (kg of BW/m^2) × pen area (4.18 m^2)/estimated final BW (3.35 kg in trial 1, and 3.29 kg in trials 2 and 3).

[2]Sx = standard error of the slope.

Table 4. Effects of stocking density on plasma total nitrites (in μM) concentration (mean $\pm$ SEM) as an indicator of nitrous oxide in heavy broilers

Stocking density[1] (kg of BW/m^2)	Trial	
	1	2
20	95 $\pm$ 5	—
25	114 $\pm$ 6	—
30	90 $\pm$ 6	70 $\pm$ 3
35	121 $\pm$ 7	74 $\pm$ 3
40	96 $\pm$ 7	74 $\pm$ 4
45	111 $\pm$ 5	75 $\pm$ 4
50	109 $\pm$ 4	—
55	101 $\pm$ 5	—
Trend analysis	Probability	
Linear	0.6	0.46
	Estimate	
Slope	0.81	1.53
Sx of slope[2]	1.54	2.03
LSD (critical values)	27.28	13.93

[1]Stocking density calculated as body mass (kg of BW/m^2) $\times$ pen area (4.18 m^2)/estimated final BW (3.35 kg in trial 1, and 3.29 kg in trials 2 and 3).

[2]Sx = standard error of the slope.

DISCUSSION

Previous work has shown that management factors and climatic conditions can have adverse effects on broilers. Siegel (1960) showed that increased population density caused the adrenal glands of chicks to hypertrophy. Later work showed that plasma CS levels increased when population density was increased such that birds were forced to compete for feeding and watering space (Pesti and Howarth, 1983; Mashaly et al., 1984; Craig et al., 1986). Smoak and Birrenkott (1986) showed that disruptions of pecking orders of broiler-type chicks resulted in elevations of the H:L ratio.

Table 5. Effects of stocking density on heterophil:lymphocyte (H:L) ratio (mean $\pm$ SEM) of heavy broilers

Stocking density[1] (kg of BW/m^2)	Trial	
	1	2 and 3
20	1.01 $\pm$ 0.16	—
25	1.01 $\pm$ 0.18	—
30	0.89 $\pm$ 0.07	1.17 $\pm$ 0.07
35	0.94 $\pm$ 0.14	1.26 $\pm$ 0.07
40	0.89 $\pm$ 0.1	1.54 $\pm$ 0.13
45	0.93 $\pm$ 0.13	1.68 $\pm$ 0.17
50	0.94 $\pm$ 0.06	—
55	1.53 $\pm$ 0.4	—
Trend analysis	Probability	
Linear	0.27	0.01
	Estimate	
Slope	0.03	0.19
Sx of slope[2]	0.02	0.07
LSD (critical values)	0.47	0.42

[1]Stocking density calculated as body mass (kg of BW/m^2) $\times$ pen area (4.18 m^2)/estimated final BW (3.35 kg in trial 1, and 3.29 kg in trials 2 and 3).

[2]Sx = standard error of the slope.

Acute exposure to both cold and hot conditions are known to affect plasma CS concentrations, H:L ratios, adrenal weights, and immunity (Thaxton et al., 1968; Thaxton and Siegel, 1972; Nir et al., 1975; Edens and Siegel, 1976; Edens, 1978; Gould and Siegel, 1985; McFarlane and Curtis, 1989).

Additionally, toxic substances, including aflatoxin and mercury, have been shown to increase adrenal weights in broilers (Thaxton et al., 1974, 1975a,b). Low protein concentrations in feed, as well as elevated dietary ascorbic acid concentrations, decreased plasma CS (Nockels et al., 1973; Pardue and Thaxton, 1984; Weber et al., 1990), whereas increased dietary vitamin A increased plasma CS (Weber et al., 1990). Finally, chronic feed restriction increased plasma CHOL concentration (Siegel, 1995).

Puvadolpirod and Thaxton (2000a,d) demonstrated that plasma concentrations of CS, GLU, CHOL, as well as H:L ratio, increased during the adaptive phase of stress in broilers. Additionally, total plasma nitrite concentration, which is a direct indicator of nitric oxide concentration, increased during oxidative stress (Stamler et al., 1992; Lancaster, 1996). Puvadolpirod and Thaxton (2000c) showed that CS concentration in broiler chicks was 1,500 pg/mL or greater at the onset of stress. They also demonstrated that plasma GLU and CHOL concentrations attained threshold concentrations of approximately 300 and 150 mg/dL, respectively, at the onset of physiological stress. Additionally, they reported that H:L ratio exhibited a 75% increase at the onset of stress.

In several in-house and field studies, we have found the CS levels of broilers to vary between 400 and 1,000 pg/mL, GLU levels to range from 200 to 250 mg/dL, CHOL to range from 100 to 200 mg/dL, and H:L ratio to range between 0.8 and 1.6. These studies have all used present-day "heavy" broiler strains that were reared under conditions not thought to evoke stress responses. Therefore, the above-stated concentrations of accepted stress indicators are indicative of nonstressed chickens. Results of the present study indicate that stocking densities from 30 to 45 kg of BW/m^2 did not cause stress at the end of the 49-d grow-out period, based on these physiological indicators.

An argument in favor of stress in the birds of trials 2 and 3 can be made based on the trend of increased H:L ratio with increases in stocking density from 30 to 45 kg of BW/m^2. However, 4 negating arguments for stress are apparent. Birds of trial 1 did not exhibit the trend of increasing H:L ratio as stocking density increased from 20 to 55 kg of BW/m^2. In previous work (Puvadolpirod and Thaxton, 2000c), the H:L ratio in birds exhibiting stress increased from 0.5 in controls to 2.76 in stressed birds. In the present study, the highest mean H:L ratio for any stocking density (45 kg of BW/m^2 in trials 2 and 3) in the 3 trials was only 1.68. The third argument is that if the birds in trials 2 and 3 were exhibiting stress, all of the other parameters would be expected to exhibit changes indicative of stress. Finally, many factors such as exposure to various microbes and chemicals can cause changes in

both granulocytic and agranulocytic white blood cells (Lucas and Jamroz, 1961).

In a companion paper, Dozier et al. (2005) demonstrated that these same stocking densities limited growth rate and feed consumption, but did not affect weights of whole carcass and breast muscle yield. Additionally, litter moisture, foot-pad lesion scores, and incidences of scratches on backs and thighs increased as stocking density increased.

Results of the present study and those of Dozier et al. (2005) indicate that stocking densities, at least from 20 to 55 kg of BW/m^2, did not cause physiological stress in broilers. However, these results are not interpreted to mean that these stocking densities do not impinge on the welfare of broilers. We agree with the conclusion of Dawkins et al. (2004), in their report of an extensive study of broilers reared under commercial conditions in the United Kingdom, that the environment had more impact on welfare than did stocking density. A complete assessment of the well-being of broilers awaits studies designed to assess several management and climatic factors, including stocking density, feeding and watering space, design and placement of feeders and waterers, temperature, humidity, air quality, and lighting (source, intensity, and duration).

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